BOARD INVITED REVIEW: Oxidative stress and efficiency: the tightrope act of mitochondria in health and disease^{1,2}

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ABSTRACT: Oxidative stress is an unavoidable consequence of aerobic metabolism. Whereas high amounts of mitochondrial reactive oxygen species (ROS) can cause oxidation, low levels play important roles in signal transduction. In a Pedigree male (PedM) broiler model of feed efficiency (FE), the low FE phenotype was characterized by increased ROS in isolated mitochondria (muscle, liver, and duodenum) with a pervasive protein oxidation in mitochondria and tissues. Subsequent proteogenomic studies in muscle revealed evidence of enhanced mitoproteome abundance, enhanced mitochondrial phosphocreatine shuttling expression, and enhanced ribosome assembly in the high FE phenotype. Surprisingly, an enhanced infrastructure would foster greater repair of damaged proteins or organelles through the autophagy and proteosome pathways in the high FE phenotype. Although protein and organelle degradation, recycling, and reconstruction would be energetically expensive, it is possible that energy invested into maintaining optimal function

of proteins and organelles contributes to cellular efficiency in the high FE phenotype. New findings in mitochondrial physiology have been reported in the last several years. Reverse electron transport (RET), once considered an artifact of in vitro conditions, now is recognized to play significant roles in inflammation, ischemia–reperfusion, muscle differentiation, and energy utilization. A topology of ROS production indicates that ROS derived from Complex I of the respiratory chain primarily causes oxidation, whereas ROS generated from Complex III are primarily involved in cell signaling. It is also apparent that there is a constant fission and fusion process that mitochondria undergo that help maintain optimal mitochondrial function and enables mitochondria to adjust to periods of nutrient limitation and nutrient excess. Understanding the balancing act that mitochondria play in health and disease will continue to be a vital biological component in health-production efficiency and disease in commercial animal agriculture.

Key words: feed efficiency, mitochondrial biology, mitochondrial dynamics, oxidative stress

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INTRODUCTION

Mitochondria, first reported by [Kennedy and Lehninger \(1949\)](#) as the major site of energy production of the cell, are believed to be the result of a commensal relationship between a methanogen and an α -proteobacterium and are possibly the single signature event that ‘...made the evolution of complex eukaryotic cells possible at all’ ([Lane, 2005](#)). Mitochondrial reactive oxygen species (ROS) have been linked to numerous metabolic conditions and diseases including aging, cancer, Alzheimer’s, Diabetes–Obesity (metabolic syndrome), ischemia reperfusion injury, inflammation, and pulmonary hypertension syndrome.

In a pedigree male (**PedM**) broiler model of feed efficiency (FE), an inextricable link between mitochondria and FE has been reported that includes lower electron transport chain (ETC) coupling with higher mitochondrial ROS production and protein oxidation in tissues obtained from animals exhibiting a low FE phenotype ([Bottje et al., 2002](#); [Iqbal et al., 2004, 2005](#); [Ojano-Dirain et al., 2004, 2005](#); [Bottje and Carsons 2009](#)). The finding of higher ROS production in low FE spurred examination of the proteogenomic landscape of muscle associated with the phenotypic expression of FE in PedM broilers (e.g., [Kong et al., 2011, 2016](#); [Bottje and Kong, 2013](#); [Bottje et al., 2017a](#)). Enrichment of autophagy and proteosome expression in the high FE phenotype muscle suggests that repair processes are enhanced in high FE ([Piekarski-Welsher et al., 2018](#), see below).

In the last decade, new aspects of mitochondrial physiology have revealed mitochondria to be dynamic organelles that move in the cell and undergo fission and fusion processes that help the cell adapt to conditions of nutrient deficiency and nutrient excess. Reverse electron transport (RET) on the mitochondrial respiratory chain or ETC, a process previously thought to be an artifact of in vitro preparations, is now known to be highly relevant for a number of physiological conditions. A topology of mitochondrial ROS production has been reported that influences whether mitochondrial ROS will cause oxidative damage or will be instrumental in signal transduction. These relatively new discoveries of mitochondrial physiology will be described in this review with emphasis on the tight rope act mitochondria play in health and disease.

PROTEOGENOMICS AND FEED EFFICIENCY

Global gene and protein expression analyses were conducted in muscle from high and low FE

PedM broiler phenotypes to gain greater understanding of how the proteogenomic landscape might differ between the phenotypes: inherent differences or differences that resulted from ROS-mediated signal transduction ([Kong et al., 2011, 2016](#); [Bottje et al., 2017a](#)). Additional FE-mitochondrial relationships were observed with the elevation of the mitoproteome in the high FE phenotype ([Kong et al., 2016](#); [Bottje et al., 2017a](#)) that provided further evidence of a link between mitochondria and FE in this PedM broiler model. The proteogenomic investigations also revealed evidence of enhanced expression of proteins requisite for ADP: ATP and creatine shuttles in the high FE mitochondria ([Bottje et al., 2017b](#); see [Figure 1](#)), enrichment of ribosome assembly and protein translation components ([Bottje et al., 2017c](#); see [Figure 2](#)), and enrichment of the autophagy and proteosomal pathways ([Piekarski-Welsher et al., 2018](#); see [Figures 3 and 4](#)) in breast muscle of the high FE PedM broiler phenotype.

[Figure 1](#), from [Bottje et al. \(2017b\)](#), is based on figures from [Brdiczka et al. \(2006\)](#) and [Schlattner et al. \(2006\)](#) showing mitochondrial creatine–phosphocreatine and nucleotide shuttling mechanisms. Also shown in [Figure 1](#) are mechanisms of energy production (oxidative phosphorylation), energy sensing, and mitochondrial biogenesis all of which were derived using data from shotgun proteomics analysis ([Kong et al., 2016](#)). Molecules in red and green indicate proteins up-regulated or down-regulated, respectively, in the high compared with the low FE PedM phenotype. Increased expression and predicted activation the electron transport chain were reported in the high FE phenotype ([Kong et al., 2016](#)) which concurred with functional data in isolated mitochondria initially reported in high FE breast muscle ([Bottje et al., 2002](#)). In [Figure 1](#), oxidative phosphorylation (OXPHOS) produces ATP that is transported sequentially across the inner mitochondrial membrane (IMM) by adenine nucleotide translocase (ANT) and across the outer mitochondrial membrane (OMM) by voltage dependent activated channel (VDAC) into the cytosol. In the cytosol, ATP would be used to carry out energy requiring reactions (cellular work), e.g., Na^+/K^+ ATPase. Phosphocreatine (PCr) is used to phosphorylate ADP in the cytosol to support “work activity” via creatine kinase (CK) in the cytosol. In the cytosol, there are two isoforms of CK, muscle and brain, that form CK dimers; the brain isoform was elevated phenotype (8.9 Fold, $P < 0.01$), whereas the muscle isoform was downregulated (1.4 fold, $P = 0.04$) in the high FE phenotype ([Bottje et al., 2017b](#)). Shuttling of creatine (Cr) and PCr between

Figure 1. Energy production and conveyance system. Depiction of energy production and conveyance system that is enhanced in breast muscle of Pedigree Male Broilers exhibiting a high feed efficiency phenotype (from [Bottje et al., 2017b](#) with modifications). The figure is based on figures from [Brdiczka et al. \(2006\)](#) and [Schlattner et al. \(2006\)](#) using proteogenomic data reported previously ([Kong et al. 2016](#)). Molecules in red and green indicate proteins or genes up-regulated or down-regulated, respectively, in the high FE Pedigree Male Phenotype. Processes shown are as follows: 1) OXPHOS: Oxidative phosphorylation and shuttling of ATP and ADP between the mitochondrial matrix and the cytosol through ANT (adenine nucleotide translocase on the inner mitochondrial membrane [IMM]) and VDAC (voltage dependent activated channel on the outer mitochondrial membrane [OMM]), 2) Biological Activity ('Work'): ATP used in cell activities such as Na⁺/K⁺ ATPase. 3) Phosphocreatine (Cr) Shuttle (Energy production and conveyance system): A mitochondrial creatine kinase (mtCK) ([Fritz-Wolf et al., 1996](#); [Khuchua et al., 1998](#)) and ANT are tethered on the IMM by cardiolipin ([Schlattner and Walliman, 2000](#)). This arrangement facilitates the transfer of phosphate from ATP to Mt-CK and to creatine (Cr) to form phosphocreatine (PCr). 4) Energy Sensing: Increased expression of AMPK (AMP activated kinase) and its catalytic subunit (PRKAA2) would stimulate 5) Mitochondrial Biogenesis by activating the transcription factor PGC1α (peroxisome proliferator-activated receptor gamma coactivator 1α).

the mitochondria and cytosol is also facilitated by VDAC on the OMM and by phosphorylation of Cr in the intramembranous space that is catalyzed by a mitochondrial CK (mtCK; [Fritz-Wolf et al., 1996](#); [Khuchua et al., 1998](#)). Both mtCK and ANT are tethered on the IMM by cardiolipin ([Schlattner and Walliman, 2000](#)), a special phospholipid with 4 fatty acid chains that are unique to mitochondria ([Pangborn, 1945](#); [Hoch, 1992](#)). The mtCK is in close proximity to ANT allowing ATP to move directly to mtCK following transport across the IMM. This arrangement facilitates the transfer of phosphate from ATP to mtCK and to creatine (Cr) to form PCr. Increased expression of AMP activated kinase (AMPK) and its catalytic subunit (PRKAA2) would function to stimulate mitochondrial biogenesis through activating the transcription factor (peroxisome proliferator-activated receptor gamma coactivator 1α (PGC1α)).

Ribosome assembly and protein translation machinery exhibited increased abundance in muscle of the high FE PedM broiler phenotype ([Bottje](#)

[et al., 2017c](#)). [Figure 2A](#) was adapted from a figure by [Wahl et al. \(2009\)](#) that summarized the cellular machinery associated with ribosomal assembly and protein translation. Using binomial distribution analysis, it was determined that virtually all of the components of this machinery were enhanced in the high FE phenotype. [Figure 2B](#) depicts the findings for increased numbers of mitochondrial ribosomal proteins (MRPs, nuclear encoded; Out of 59 transcripts for mitochondrial ribosomal proteins that were detected, 55 were higher in the high FE compared with the low FE phenotype PedM broiler; the binomial distribution *P* value was 8×10^{13} [[Bottje et al., 2017c](#)])¹ and mitochondrial tRNA (mt-tRNA encoded by mitochondrial DNA) that are required for the construction of mitochondrial ribosomes and mitochondrial protein (mt-Protein) synthesis that would support the increased expression of the mitoproteome in the high FE phenotype reported in [Kong et al. \(2016\)](#).

Protein synthesis and protein turnover are energetically expensive processes. Thus, it is surprising

Figure 2. Ribosome assembly and protein translation processes. Ribosome assembly and protein translation processes were enriched in the high feed efficiency (FE) pedigree male broiler phenotype reported in Bottje *et al.*, (2017c). Components shown in pink or red were enriched in the high FE phenotype. (A) RNA polymerases initiate transcription in the nucleoplasm or in the nucleolus. Small nuclear (sn) ribonucleoproteins (SNP) and heterogeneous (hn) SNP play roles in nucleolar ribosomal RNA (rRNA) synthesis as well as in messenger RNA (mRNA) synthesis in conjunction with spliceosome activity responsible for cleaving on introns from the pre mRNA molecule. Pre-ribosomes as well as tRNAs and mRNAs are exported out of the nucleus into the cytoplasm. In the cytoplasm, ribosomes assemble into the 40S and 60S subunits where protein translation takes place in conjunction with delivery of amino acids by tRNA. The figure is adapted from Figure 1 (p. 702) of Wahl *et al.* (2009). (B) Nuclear-encoded mitochondrial ribosomal proteins (MRPs) are imported into the mitochondria through the outer mitochondrial membrane transport proteins (TOMM) and through the inner mitochondrial membrane proteins (TIMM). In RNAseq analysis data generated by Bottje *et al.* (2017a), 55 of 59 MRPs were numerically or significantly higher in the high FE phenotype compared to the low FE phenotype resulting in a binomial distribution P value of 8×10^{-13} (shown in table 3, Bottje *et al.*, 2017c). There were also significant binomial distribution P values of 0.0029 and 0.0018 for TIMM and mitochondrial tRNA, respectively.

that expression of autophagy and proteasome pathways was enriched in the high FE phenotype (Figures 3 and 4). A list of genes depicted in Figures 3 and 4 is provided in Table 1. This is especially surprising since the low FE phenotype exhibited pervasive oxidation in muscle mitochondria and in liver, duodenal, heart, and muscle homogenates (Bottje and Carstens, 2009). Based on these data, it was hypothesized that maintaining optimal functionality of proteins is paramount for optimal cellular efficiency (Piekarski-Welscher *et al.*, 2018).

MITOCHONDRIAL TIGHTROPE IN HEALTH AND DISEASE

Mitochondrial Dynamics

Initial electron micrographs of mitochondria suggest that kidney-shaped image of mitochondria as being a static organelle (Lehninger *et al.*, 1993; p. 39). Electron tomography (Mannella,

2000) provided a 3-dimensional view of a sausage-like appearance of mitochondria extending through the cytosol with intimate connections to endoplasmic reticulum that could facilitate communication with the rest of the cell, protein transfer and signaling. It is now apparent that mitochondrial fission and fusion events regulate mitochondrial function over a broad spectrum of available energy sources that can serve to cull defective organelles from the “mitochondrial herd” (see reviews by Hoppins *et al.*, 2007; Archer, 2013; Liesa and Shirhai, 2013; Rambold and Pearce, 2018). The reader is encouraged to follow this link on Google (<https://www.youtube.com/watch?v=CIXY-Ns5vks>—source New England Journal of Medicine [2014]) associated with Archer (2013) that clearly shows in short-microscopy videos the dynamic nature of mitochondria in several different cell types. In addition, it is clear that intimate connections of mitochondria with other organelles in the cell including the nucleus, endoplasmic reticulum, and lysosomes help in movement of proteins and as

Table 1. Names of genes or proteins that are abbreviated in the text and figures

Abbrev.	Genes or protein name
AMBRA	autophagy and beclin 1 regulator 1
AMPK	AMP activated kinase
ATG	autophagy related
BECN1	Beclin 1
DRAM1	DNA damage regulated autophagy modulator
EPG5	ectopic P-granule autophagy modulator
LC3B	Microtubule-associated protein 1A/1B-light chain 3
MAP1LC	microtubule associated protein light chain
mTOR	mechanistic target of rapamycin
NBR1	NBR1, autophagy cargo receptor
PEMT	phosphatidyleanolamine N-methyltransferase
PINK1	PTEN induced putative kinase 1
PIP3	phosphoinositide 3- phosphate
PSMA	proteasome subunit alpha
PSMB	proteasome subunit ATPase
PSMC	Proteasome 26 subunit ATPase
PSMC3IP	PSMC interacting protein
PSMD	proteasome non-ATPase regulatory subunit
PSMF	proteasome inhibitor subunit 1
PSMG	Proteasome assembly chaperone
RUBCN	RUN and cysteine rich domain containing beclin 1
STX17	Syntaxin 17
TSNARE	t-SNARE domain containing
ULK	unc- like autophagy activating kinase
UVRAG	UV radiation resistance associated
VPS	Vasculuar sorting protein
WIPI	WD repeat domain phosphoinositide interacting

pathways for cell signaling to optimize cell function (Rambold and Pearce, 2018).

Liesa and Shirhai (2013) reviewed fission and fusion events in a mitochondrial life cycle that is depicted in Figure 5. With excess nutrients, mitochondria divide (fragment) and become less efficient in ATP production to burn off excess nutrients to lower the amount of energy substrate in the cell. In this fragmentation (fission) process, the endoplasmic reticulum targets the mitochondria with proteins, including mitofissin, dynamin, and dynamin-like 120 kDa—protein (OPA-1 short), that split the mitochondria in two. Fission is part of the mitochondrial life cycle that allows mitochondria to adapt to different amounts of available nutrients. In a nutrient limiting environment (e.g., starvation), mitochondria become elongated and more efficient in producing ATP from energy substrates. In the life cycle, dysfunctional mitochondria are destined for removal by mitophagy (Figure 5), thereby raising the overall functioning of the remaining mitochondrial population (“mitochondrial herd”) in cells.

Signal Transduction and Reverse Electron Transport

Instead of simply being a major site of oxidative stress, mitochondria are increasingly

Figure 3. Feed efficiency and the autophagy pathway. Evidence for autophagy pathway enrichment was reported by Piekarski-Welsher *et al.* (2018). Genes shown in dark red were differentially expressed as determined by reverse transcriptase polymerase chain reaction (RT-PCR). Genes in pink and green that were higher or lower, respectively, in breast muscle of the high FE compared to the low FE Pedigree Male phenotype. Vacuolar sorting protein (VPS) genes are shown as bars for simplicity. The figure was based on one provided in Piekarski *et al.* (2015) with modifications.

Figure 4. Feed efficiency and proteasome subunit expression. A schematic of the fully assembled 26S Proteasome is shown on the left side of the figure (from [Piekarski-Welsher et al., 2018](#)). This schematic is based on a figure reported by [Tanaka et al. \(2009\)](#). Subunits filled with pink represent those that had higher expression (numerical or significant) from transcriptomic data obtained by [Bottje et al., \(2017a\)](#) (shown in a list on the right side of the figure) in breast muscle of the high FE pedigree male (PedM) broiler phenotype whereas those in green had higher expression in the low FE PedM broiler phenotype. Similarly, pink and green asterisks indicate higher expression in high and low FE PedM broiler phenotypes, respectively, from proteomic data obtained by [Kong et al. \(2016\)](#). The binomial *P* values shown at the top of the molecule lists indicate a significant skew favoring greater abundance of proteasome subunits in the high FE phenotype.

recognized to play many complex roles linked to cellular homeostasis. [Finkel \(1998\)](#) proposed that H_2O_2 stimulated several signaling pathways by oxidation of cysteine moieties of key regulatory proteins that are modulated by peroxiredoxin and other antioxidants ([Rhee et al., 2005](#)). Low levels of mitochondrial ROS contribute to important mitochondrial-mediated signal transduction mechanisms ([Murphy et al., 2011](#); [Finkel, 2012](#)) in a process designated as “mitochondrial hormesis (mitohormesis)” ([Barbieri et al., 2013](#)). Furthermore, although mitochondrial ROS generated from Complex I is associated with oxidative stress, ROS generated from Complex III is primarily involved in signal transduction ([Sena and Chandel, 2012](#); [Bleier et al., 2015](#)). Up to 11 sites of mitochondrial ROS production have been identified ([Goncalves et al., 2015](#); [Brand,](#)

[2016](#)). Reverse electron transport (**RET**) in the mitochondria is now recognized to be vital to a number of physiological conditions (see review by [Scialo et al., 2017](#)).

Specific Protein Targets of Mitochondrial ROS

Mitochondrial ROS produced by CI are exclusively released into the mitochondrial matrix, whereas ROS (superoxide) produced at the ubiquinone-CIII site are confined to the intermembranous space ([Han et al., 2001](#); [St. Pierre et al., 2002](#), [Muller et al., 2004](#)). [Bleier et al. \(2015\)](#) hypothesized that ROS generated at CI, which is in close proximity to mitochondrial DNA and redox sensitive proteins in the ETCs, would mostly cause oxidation, whereas ROS generated at CIII would primarily participate in signal transduction

Figure 5. The mitochondrial life cycle. In the mitochondrial life cycle, mitochondria are able to raise or lower energetic efficiency through biochemical and conformation changes in response to a range of nutrient levels from little or none (e.g., starvation, top) to excess nutrients (e.g., obesity, bottom; see [Liesa and Shirihai, 2013](#)). In fusion events (1) two daughter cells can combine to form a single mitochondria. With nutrient limiting conditions that occurs in an extreme case of starvation, but might also be hypothesized to occur in high energy demand conditions (e.g., muscle contraction, rapid growth of tissue), the mitochondria elongate and become more efficient in utilizing energy substrates. Once nutrient levels are no longer limiting, the mitochondria will return to a more normal size. Parts of the mitochondria may experience oxidative damage and increased mitochondrial ROS production with concomitant oxidation of mt-DNA. When this occurs, the endoplasmic reticulum will present specialized proteins (mitofissin, OPA-1, dynamin) to initiate mitochondrial fission (2). In this quality control process, the damaged or poorly functioning mitochondria with depolarized inner mitochondrial membrane will split from the optimal functioning mitochondria. If the mitochondria recovers from depolarization it will be returned into the mitochondrial life cycle (3). If mitochondria experience sustained depolarization of the membrane (4), it will be targeted for degradation by mitophagy (5). In response to excess nutrients (e.g., obesity), mitochondria will fragment (6) and become less efficient in producing ATP. Associated with this condition is an increase in mitochondrial ROS production that can have deleterious consequences to the cell. When cell nutrients levels decline, the mitochondria will return to the mitochondrial life cycle.

reactions. Using 2D gel electrophoresis with fluorescent detection, [Bleier et al. \(2015\)](#) identified specific proteins that were oxidized by ROS produced at CI (by RET) differed from proteins oxidized by ROS generated at CIII (by forward electron transport) in isolated rat heart mitochondria. They concluded that there was a distinct difference in target protein oxidation depending on site of ROS production and hypothesized that these proteins might “...serve as biomarkers to study the generator-dependent dual role of mitochondrial ROS in redox signaling and oxidative stress.”

Reverse Electron Transport Optimizes Nutrient Utilization

[Guaras et al. \(2016\)](#) demonstrated that a RET mechanism enables mitochondria to efficiently use different energy sources entering the ETC. In

this mechanism, the ratio of reduced coenzyme Q (CoQH_2) to oxidized CoQ, that transfers electrons from CI to CIII and CII to CIII, acts as a sensor to optimize energy utilization by stimulating RET. The ETC is composed of 5 multiprotein complexes (see above) that can be arranged into supercomplexes consisting of CI+CIII+CIV and CI+CIII (Acin-Perez et al., 2008; [Enriquez, 2016](#)). It is proposed that this supercomplex arrangement creates 2 functional pools of CIII: one is associated with CI and the other is not associated with CI ([Lapiente-Brun et al., 2013](#)). When the energy source switches from glucose to fatty acids, ROS produced by RET degrade CI, enhancing mitochondrial β -oxidation of fatty acids. ETC remodeling is a response to ubiquinone acting as a metabolic sensor that positions the mitochondria to efficiently oxidize different nutrients as they become available in the cell.

Reverse Electron Transport Affects Macrophage Function

Mitochondria enable macrophages to quickly shift from an “attack and destroy” cell to a “heal and rebuild” cell needed for tissue repair after the initial foreign invasion or insult. In a bacterial infection, ETC reorganization occurs in macrophages in which CI activity decreases and CII activity increases (Garaude *et al.*, 2016). As demonstrated by Mills *et al.* (2016), the ability of macrophages to switch between these very different functional phenotypes occurs through a shift in mitochondrial energy production (from OXPHOS to glycolysis) concomitant with an increase in membrane potential that helps drive RET-derived ROS production and pro-inflammatory cytokine production. This redirection of mitochondrial function was related to succinate accumulation and succinate dehydrogenase activity. Inhibition of pro-inflammatory cytokines in macrophages was achieved by 1) treating cells with dimethyl malonate to prevent succinate oxidation, 2) treating cells with rotenone to block RET from succinate, and 3) preventing CoQ reduction. These findings clearly indicate that ROS generated by RET from succinate is essential in initiating an inflammatory response in macrophages.

Reverse Electron Transport and Ischemia–Reperfusion Injury

When blood supply is interrupted (e.g., heart attack, stroke, or severe blood loss), and followed by blood flow restoration, oxidative damage of tissues occurs from a hyper-immune response and a burst of mitochondrial ROS production. Mitochondrial ROS generated during reperfusion was considered mainly a nonspecific response to an increase in oxygen supply to the tissue (e.g., Murphy and Steenbergen, 2008). However, Chouchani *et al.* (2014) demonstrated that accumulation of succinate during ischemia drives mitochondrial ROS production during reperfusion as a result of RET from Complex II to Complex I. Ischemic damage of heart and brain tissue was prevented by rotenone (that blocks RET from CII to CI) and by inhibition of succinate dehydrogenase with dimethyl malonate.

Reverse Electron Transport and Muscle Differentiation

Many myogenic regulatory factors are expressed that promote differentiation of satellite

cells into mature myoblasts and myotubes in avian species (see Velleman and McFarland, 2015). Hydrogen peroxide produced by NADPH oxidase (NOX) is instrumental in differentiation of cardiac cells and muscle cells (Piao *et al.*, 2005; Buggisch *et al.*, 2007). Since H_2O_2 is lipid soluble, it readily crosses membranes in the various cell compartments. Using mitochondria specific antioxidants (MitoQ and MitoTEMPOL), it was determined that suppression of muscle differentiation was concomitant with decreased mitochondrial ROS production (Lee *et al.*, 2011). This response was not due to inhibition of electron transport or ETC activity since these antioxidants had no effect on ATP production. During differentiation, there was an increase in mitochondrial content and increased expression and activity of CI. Treating cells with small inhibitory RNA or blockade of electron transport in CI inhibited muscle cell differentiation. It was concluded that superoxide produced by RET at CI was converted to H_2O_2 by MnSOD that stimulated muscle differentiation in response to NF- κ B activation (Lee *et al.*, 2011).

AGING, COMPLEX I, AND ROS PRODUCTION

Examination of the mitoproteome that focused on CI assembly and stabilization produced a new insight into the role of mitochondria in aging (Miwa *et al.*, 2014). CI is the largest complex of the respiratory chain containing ~40 proteins with 2 arms: one that extends into the mitochondrial matrix and a second arm that is found primarily within the inner mitochondrial membrane. The mitoproteome of young animals had reduced abundance of proteins in the matrix arm of CI in long-lived animals, but there was no difference in the abundance of the membrane arm of CI between short-lived and long-lived groups. Disruption of CI assembly reduced oxidative metabolism and elevated mitochondrial ROS production. Disruption of CI assembly resulted in premature senescence in primary hepatocytes. Miwa *et al.* (2014) hypothesized that decreased abundance of proteins in the catalytic component of CI improves assembly of the enzyme complex and improves substrate utilization while reducing mitochondrial ROS production and increasing longevity.

SUMMARY

It is apparent that mitochondria occupy a vital role in health and disease. High levels of

Figure 6. Tightrope act of mitochondria. Mitochondria are continually walking a tightrope in many processes outlined in this review. (A) The mitochondrial tightrope walker (Mito Man) is shown balancing supply of energy from nutrients with energy use (work) and a certain amount of waste (mostly proton leak). Also, shown are the important roles that mitochondria play in redox balance of the cell and ability to shift forward electron flow to reverse electron flow (see text for details). (B) In this figure, excess nutrient availability is the result of an imbalance in energy supply. (C) To offset the imbalance, Mito Man decreases mitochondrial efficiency through mitochondrial fragmentation and increased proton and electron leak. The balancing act depicted in this figure was modified from one shown in [Liesa and Shirihai \(2013\)](#).

mitochondrial ROS production can cause oxidative damage associated with etiology of numerous metabolic disease, yet low levels of mitochondrial ROS are vital in redox signaling to enable the cell to respond appropriately to a wide variety of challenges. Forward electron flow along the electron transport chain is necessary to generate ATP by oxidative phosphorylation, but reverse electron flow enables the mitochondria to optimize energy substrate utilization in the cell. Mitochondria also undergo continual fission and fusion reactions to optimize function of mitochondria in response to changes in nutrient levels. Proton leak can attenuate mitochondrial ROS production but represents a mitochondrial inefficiency as the mitochondrial membrane potential is dissipated without concomitant synthesis of ATP. Reverse electron flow leading to oxidative damage is detrimental to tissues undergoing reperfusion after ischemia, but reverse electron is required for macrophages to destroy foreign material through ROS production.

The tightrope act that mitochondria undergo in processes outlined in this review is shown in [Figure 6](#). The tightrope walker (Mito Man) is shown balancing supply of energy from nutrients with energy use (work) and a low amount of waste (mostly proton leak). The Mito Man is also shown carrying other important roles in redox balance along with the ability to shift forward electron flow to reverse electron flow depending on nutrient load. Excess nutrient availability results in an imbalance in energy supply that is offset by wasteful processes that is facilitated by mitochondrial fragmentation and increased proton and

electron leak. Thus, the tightrope act of mitochondria is a central component to health and disease.

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